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THE EVOLUTION OF THE ALARM-DEFENSE SYSTEM IN THE FORMICINE ANTS

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In the social insects, alarm communication and colony defense are inextricably combined, for the reason that the workers rely heavily on chemical secretions to accomplish both functions. In many instances, the same substances serve simultaneously to repel invaders and to alert nestmates to danger (Regnier and Wilson 1968; Blum 1969; Moore 1969). For this reason it is useful to refer to a combined "alarm-defense system" of a given species (Wilson 1971).

Because the secretions involved are typically plentiful and relatively easily identified, we saw in them the opportunity to conduct one of the first comparative studies of an exocrine system used in chemical communication. The subfamily Formicinae was selected as the most favorable group of ants on which to concentrate. Workers of this phylogenetically advanced taxon store large quantities of alarm-defense substances in their glandular reservoirs, and abundant colonies of many phyletic stocks are available in the north temperate zone. Of equal importance, the phylogeny of much of the group, in particular the genera *Lasius* (Wilson 1955), *Acanthomyops* (Wing 1968), and *Formica* (Buren 1968) are already comparatively well understood on morphological and zoogeographic grounds. Our ultimate goal is to elucidate the evolution of a pheromone system. Earlier accounts were concerned with the alarm-defense systems of the genera *Acanthomyops, Formica,* and *Lasius* (Regnier and Wilson 1968, 1969, 1971; Bergström and Löfqvist 1970). In this, the first report to attempt a phylogenetic review of the systems, we will add data on a diversity of other genera within the subfamily Formicinae and present our interpretation of the adaptive patterns thus far discovered.

EVOLUTIONARY TRENDS

The evolution of a chemical communication system inevitably consists of correlated alterations in morphological, behavioral, and biochemical characters. The alarm-defense system of the Formicinae has been demonstrated to be based upon a limited number of substances of low molecular weight, primarily terpenes, alkanes, and ketones, which are stored in the reservoirs of two kinds of exocrine glands—the paired mandibular glands, located in the anterior part of the head cavity; and Dufour's gland, located at the

rear of the abdominal cavity (fig. 1). Adaptive change has been accomplished by shifting the nature and quantities of the substances, the stimuli required for their discharge, and the form of the response to them. The list below provides an evaluation, character by character, of the trends of variation within the Formicinae. In obedience to the standard procedures of phylogenetic systematics, an attempt is made to identify primitive and

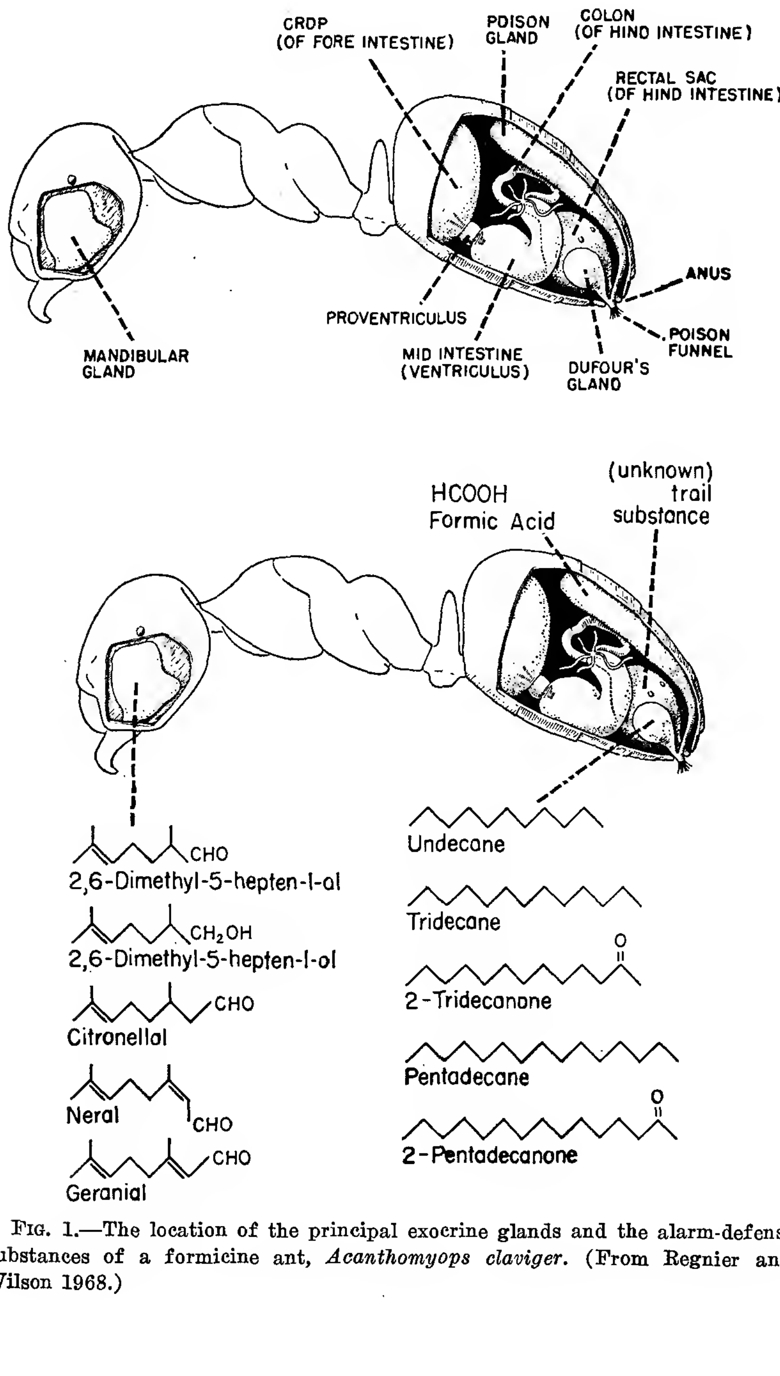


Fig. 1.—The location of the principal exocrine glands and the alarm-defense substances of a formicine ant, *Acanthomyops claviger*. (From Regnier and Wilson 1968.)

derived conditions. In general, the primitive condition is judged to be the one that is widespread—preferably, universal—in those groups within and beyond the Formicinae which, on fossil and comparative anatomical evidence, are regarded closest to the ancestral stocks.

1. *Size of the mandibular gland.*—The mandibular gland has been examined only sporadically in the Myrmicinae (*Aphaenogaster, Crematogaster, Pogonomyrmex, Solenopsis*), Dolichoderinae (*Tapinoma*), and Formicinae (*Acanthomyops, Formica, Lasius*). The gland is much larger in *Acanthomyops* than in these other genera (fig. 1). Since *Acanthomyops* is clearly derived from *Lasius* (Wilson 1955; Wing 1968), large mandibular gland size can be safely regarded as a derived state.

2. *Chemistry of the mandibular gland secretions.*—From one ant taxon to another the mandibular gland contents vary greatly and in no clearly perceptible pattern: farnasene and pentadecane in *Aphaenogaster,* methylheptanone in *Pogonomyrmex,* 3-octanone in *Crematogaster,* and, within the Formicinae, a wide variety of acyclic terpenes. Cyclic terpenes, or furans, have been reported from the formicine *Lasius fuliginosus.* In many of the formicine species we have tested (table 1), however, no substances attributable to the mandibular gland were detected. It is entirely possible, and even probable, that components are nevertheless present in nanogram quantities or less. The best judgment we can make at this time is that the large quantities of citronellal and other terpenoids in *Acanthomyops* and *Lasius* subgenus *Chthonolasius* are a derived condition, and that the furans of *L. (Dendrolasius) fuliginosus,* being known only from this taxon, which is itself a parasitic form derived from *Lasius (Lasius),* are also derived.

3. *Anatomy of Dufour's gland.*—This gland, the "accessory gland" of the older literature, is intimately associated with the poison gland. Its reservoir opens at the base of the sting. In the course of our study we learned that the form of the gland varies greatly from species to species and can serve as a valuable taxonomic character at both the generic and species level (see fig. 2). In ants generally, including the more primitive members of the myrmecioid complex outside of the Formicinae, namely the genera *Myrmecia* and *Aneuretus,* the gland is small to moderate in size and single-lobed (Robertson 1968; Wilson, unpublished observations). The bilobed condition of the gland in the Formicinae is therefore apparently derived. The enormous size of the gland in certain species of the *Formica sanguinea* group (subgenus *Raptiformica*)—namely, *F. pergandei* and *F. subintegra*—is certainly derived. The members of the *F. sanguinea* group are slave-makers and therefore specialized parasites. Furthermore, at least one species (*F. rubicunda*) retains the smaller gland size seen in other species groups of *Formica.*

4. *Chemistry of the secretions from Dufour's gland.*—Within the Formicinae, Dufour's gland produces undecane and higher odd-numbered alkanes, which serve to spread and to enhance the penetration of formic acid originating from the poison gland. These same substances, along with their equivalent ketones, also function as alarm pheromones (Regnier and Wilson 1968, 1969). Because these alkanes and ketones are universally dis-

TABLE 1

CONCENTRATION OF INDIVIDUAL VOLATILE COMPONENTS (% OF BODY WEIGHT $\times 10^{-2}$)

	Undecane	2-tri-decanone	2-penta-decanone	Decyl acetate	Dodecyl acetate	Tetradecyl acetate	Citro-nellal	Citro-nellol	*Trans*-citral	*Cis*-citral
Lasius pallitarsus (=*sitkaensis*)	21.0	...	...	...	...	...	...	...	...	...
*L. alienus**	11.0	6.0	0.3	...	...	...	tr	0.1	...	...
L. neoniger	18.0	12.0	...	...	...	...	...	...	...	...
*L. flavus**	6.5	1.5	...	...	...	...	...	...	...	...
L. nearcticus	24.0	10.0	0.8	...	...	...	...	...	...	...
*L. umbratus**	50.0†	26.0†	1.0†	...	...	...	tr	1.8‡	...	...
*L. speculiventris**	36.0	11.0	3.2	...	...	...	2.0	tr	...	...
*Acanthomyops claviger**	11.0†	8.4†	2.0†	...	...	...	19.0‡	...	tr‡	tr‡
A. latipes	35.0	20.0	3.2	...	...	...	41.0‡	...	14.0‡	2.1‡
A. subglaber	6.0	0.6	0.6	...	...	...	1.4	...	tr	tr
Formica neogagates	50.0	25.0	0.8	...	...	...	...	...	...	...
F. subsericea	8.2†	...	...	...	...	...	...	...	...	...
F. schaufussi	6.5	...	...	...	...	...	...	...	...	...
F. rufa	7.5	...	...	...	...	...	...	...	...	...
F. exectoides	15.0	...	...	...	...	...	...	...	...	...
F. rubicunda§	7.6	1.9	...	...	...	...	...	...	...	...
F. pergandei	5.7†	...	...	35.0†	92.0†	44.0†	...	...	...	...
F. subintegra	...	...	...	760.0†	290.0†	280.0†	...	...	...	...
Camponotus novaboracensis	2.9‖/3.6#	...	...	...	...	...	...	...	...	...
C. pennsylvanicus	19.0‖/27.0#	...	...	...	...	...	...	...	...	...
C. americanus	24.0†	...	...	...	...	...	...	...	...	...

* Compounds in these species were identified by combination gas chromatography-mass spectrometry. Compounds in all other species were identified by comparison of their gas chromatographic retention times with those of synthetic standards.

† Concentrations given have been shown to be in the Dufour's gland by gas chromatographic analysis of the excised glands.

‡ These compounds have been localized in the mandibular gland by gas chromatographic analysis of the excised gland.

§ This species also produces nonane in approximately the same concentration as undecane.

‖ Concentrations given are for minor workers.

\# Concentrations given are for major workers.

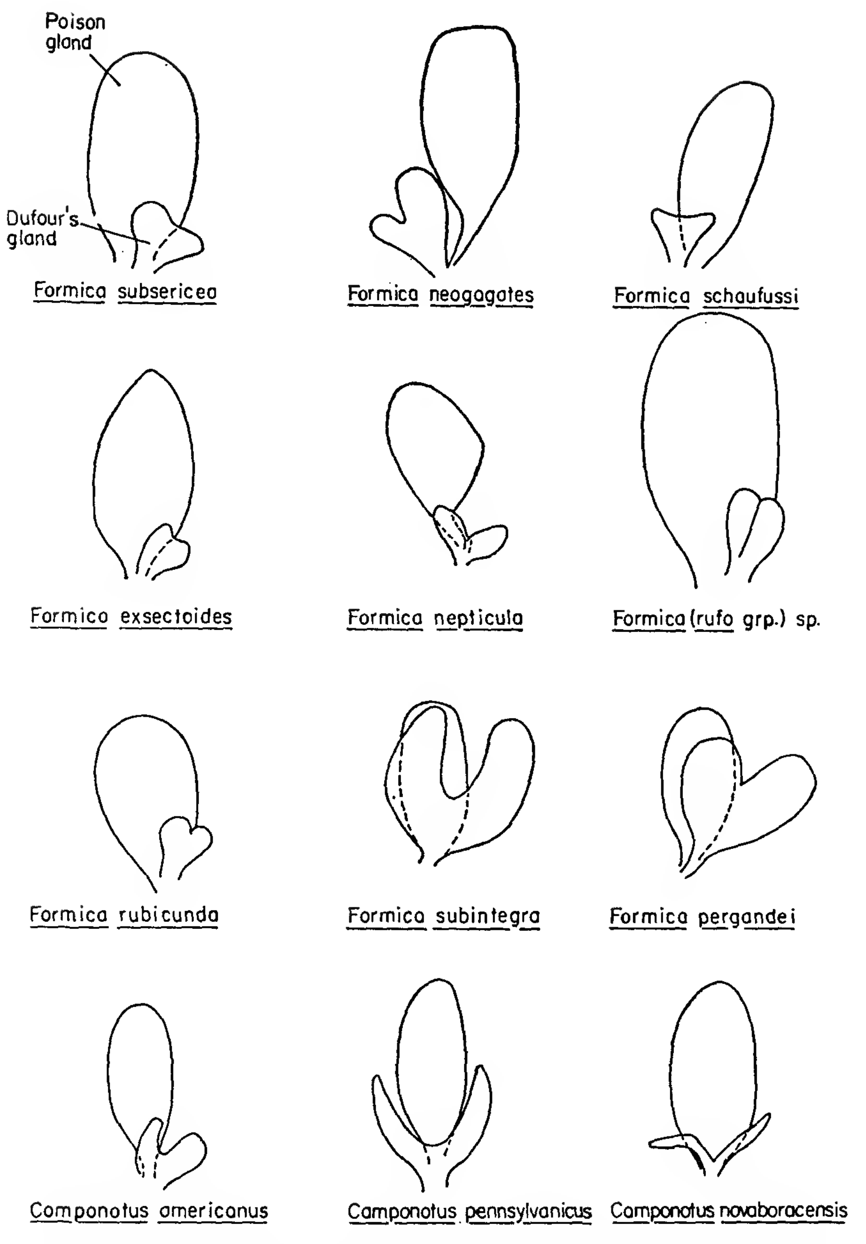


FIG. 2.—The poison and Dufour's glands of selected species of formicine ants. As these outline drawings indicate, the form of the Dufour's gland varies sufficiently from species to species to serve as a taxonomic character. All of the specimens illustrated are from eastern Massachusetts. The figures are not drawn to scale.

tributed through the species we have examined (see table 1), we interpret their presence to be a primitive trait within at least the higher Formicinae. On the other hand, the two slave-making species of *Formica* (*F. pergandei*, *F. subintegra*) with enlarged Dufour's glands have been found to manufacture decyl, dodecyl, and tetradecyl acetates. Bergström and Löfqvist (1968; 1970) have also reported the presence of decyl, undecyl, and dodecyl acetates in the European slave-making species *F. sanguinea* and, together with variable amounts of several homologs, in the free-living *Lasius niger*. We consider the production of these acetates to be a derived state. Bergström and Löfqvist (1970) have also detected the presence of two lactones (4-hydroxyhexadec-9-enolide and 4-hydroxyoctadec-9-enolide) and a related acid (4-hydroxyoctadec-9-enoic acid) in the abdomens of *Lasius flavus* workers. These substances are believed to originate in Dufour's gland. Whatever their source, we interpret them as probably representing a derived state in evolution.

5. *Nature of the alarm-defense reaction.*—The response of worker formicines to disturbances of their nest varies enormously among species. In general, species with small mature colonies, or colonies that are diffusely distributed (and thus at low population densities) scatter quickly when the nest is disturbed. Risks are taken to retrieve all of the brood and some of the workers attack the intruder, but the overall response to a major disturbance is a quick retreat. Few studies have been made of the alarm systems of such species. The workers of *Lasius alienus* have relatively low response thresholds to their own alarm substances, varying in order of magnitude from 10^7 to 10^{11} molecules/cubic centimeter. They are easily triggered into excited and prolonged running. Although some of the alarmed workers orient briefly toward the odor source, the majority run in an erratic, seemingly aimless pattern. The result is a rapid scattering of the colony (Regnier and Wilson 1969). We proposed to designate this extreme form of response as "panic alarm." In contrast, species that form large, compact colonies tend to respond to major disturbance by aggressive behavior. The workers are excited, and many run in erratic patterns, but most move deliberately toward points of disturbance and attack alien objects without hesitation. The colony, in short, engages in a "stand and hold" defense. In the few cases where chemical communication of such species has been examined, namely in *Acanthomyops* and the members of the *Formica sanguinea* group, the workers are strongly attracted by their own alarm pheromones. We suggest that this overall pattern of response be called "aggressive alarm." At the present time it is impossible to judge whether panic alarm or aggressive alarm is the primitive state within the Formicinae. The character is probably a very labile one, subject to reversal as colony size and density are altered during evolution. The species of *Myrmecia*, the most primitive living genus of the myrmecioid complex and hence closest in line to the ancestry of the Formicinae, have large, compact colonies that respond with aggressive alarm. On the other hand, *Myrmecorhynchus* of Australia and *Gigantiops* of South America, which are

among the most primitive living Formicinae, display the panic form of alarm (Wilson, personal observations). We suspect that among the higher Formicinae, particularly within *Formica, Lasius,* and their satellite genera, panic alarm is primitive and aggressive alarm is derived.

ADAPTIVE PATTERNS

Our survey of the Formicinae has so far revealed two kinds of major adaptive changes in the alarm-defense system (see fig. 3). The first of these is culminated in the genus *Acanthomyops.* This exclusively Nearctic taxon was derived from *Lasius,* and at least some of the species are temporary social parasites on free-living species of that genus. The alarm-defense

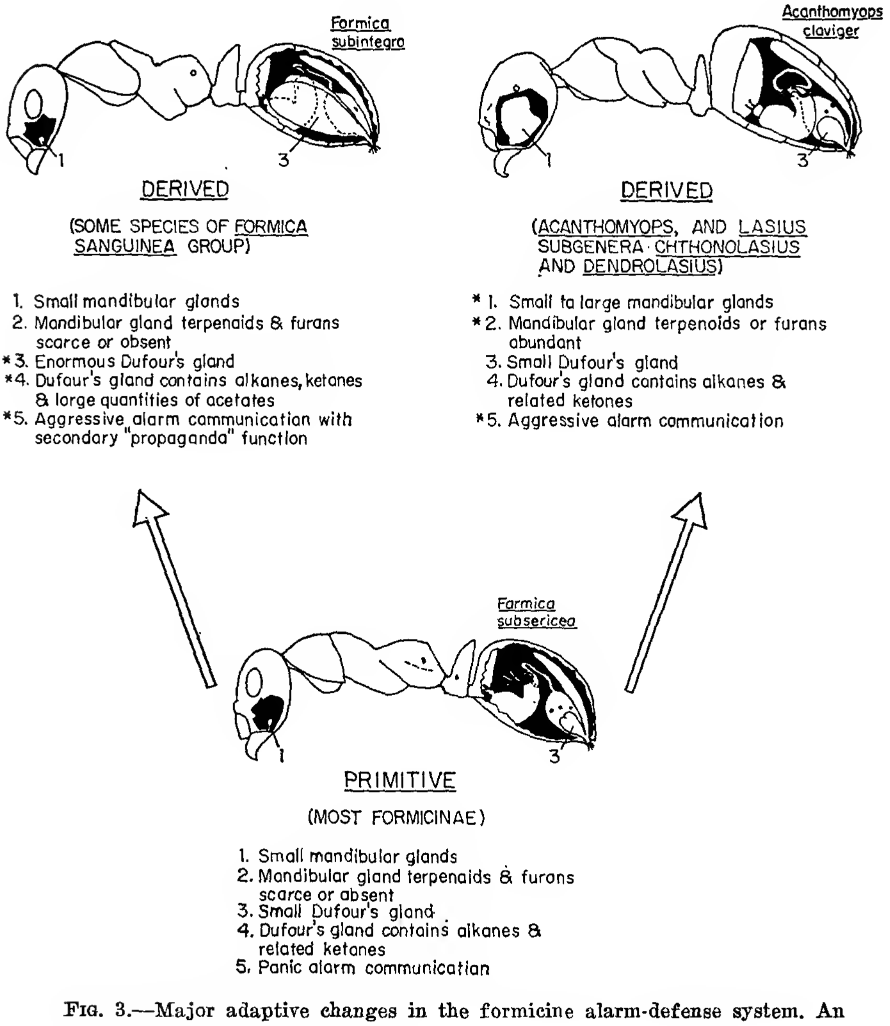
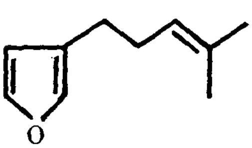


FIG. 3.—Major adaptive changes in the formicine alarm-defense system. An asterisk indicates a new character state.

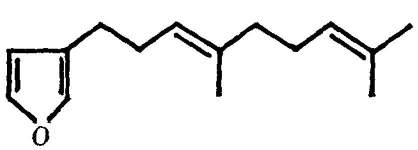
system of *Acanthomyops* is distinguished by a hypertrophy of the mandibular gland, which produces large quantities of citronellal and two isomers of citral. Disturbed colonies smell strongly of these substances, and for this reason the *Acanthomyops* are often referred to as "citronella ants." The terpenes function in part as defensive substances in combination with the undecane-formic acid mixtures expelled from the poison funnel of the abdomen (Ghent 1961; Regnier and Wilson 1968). They also serve in conjunction with C_{11}—C_{13} substances from Dufour's gland (undecane, tridecane, tridecanone) as efficient alarm substances. The behavioral threshold concentration of all these substances is somewhat higher than in *Lasius alienus,* ranging in order of magnitude from 10^{10} to 10^{12} molecules/cubic centimeter. The active space of the pheromones (i.e., the space within which the substances are at or above threshold concentration) spreads rapidly and extends over a distance of centimeters. The workers respond to the signal with a markedly aggressive form of alarm. They orient toward the odor source, apparently by moving up the concentration gradient, and attack alien objects in their path. Colonies of *Acanthomyops* tend to be very large, containing thousands or tens of thousands of workers, and are often densely concentrated in narrow subterranean galleries and chambers. They also depend on "herds" of symbiotic root homopterans, which cannot be easily rescued when the nests are disturbed. We postulate that these circumstances have led to the evolution of the aggressive alarm response. In other words, it is to the advantage of the colony to stand and fight, at least for awhile, rather than to try to make an immediate retreat.

On morphological and behavioral grounds the subgenus *Chthonolasius* of *Lasius* is considered to occupy an intermediate phylogenetic position between *Lasius* subgenus *Lasius* and *Acanthomyops.* The species of *Chthonolasius,* like some of the members of *Acanthomyops,* are temporary social parasites on free-living species of the subgenus *Lasius.* It is therefore interesting to note, from the data presented in table 1, that the chemical composition of the *Chthonolasius* mandibular glands is also intermediate between the compositions of *Lasius (Lasius)* and *Acanthomyops.* The *Chtonolasius* are *umbratus* and *speculiventris.*

The subgenus *Dendrolasius* of *Lasius,* a third group of temporary social parasites on *Lasius (Lasius)* and one that has a largely independent history, has evolved an alarm-defense system parallel to that of *Acanthomyops.* But instead of increasing the quantity of terpenes, the *Dendrolasius,* as exemplified by *L. (D.) fuliginosus* of Europe, have "invented" the following two terpenoid furans, which the workers produce in large quantities in their mandibular glands (Bernardi et al. 1967):



Perillen



Dendrolasin

Dendrolasin is far more abundant than perillen, which possibly exists only as a short-lived precursor with little functional significance. Dendrolasin is an effective defensive substance, but according to Maschwitz (1964) it does not serve as an alarm pheromone. *Lasius (D.) fuliginosus,* like *Acanthomyops claviger,* forms large, compact colonies that nest in confined places.

The second major adaptive step is the one taken by certain members of the *Formica sanguinea* group. These species, exemplified so far by *F. pergandei* and *F. subintegra* of the United States, have grotesquely enlarged Dufour's glands. The principal components of the glands are decyl acetate, dodecyl acetate, and tetradecyl acetate, compounds so far unknown in other kinds of ants except the closely related *F. sanguinea* of Europe. These substances are present in surprisingly large quantities. One *F. subintegra* worker contains a total of 700 μg, or approximately one hundred times all of the volatiles present in a worker of comparable size belonging to ordinary species of *Formica.* During slave raids on colonies of other species of *Formica,* the workers spray copious amounts of the acetates on any defenders who stand up to them. The acetates function as alarm pheromones for the attacking workers, increasing their degree of excitement, and attracting them to the combat sites. They also alarm the defending workers, which are more apt to be disoriented and scattered as a result. This is especially true when the victim species are characterized by the panic form of alarm. In fact, the acetates are functionally "super-pheromones," since they are (1) dispensed in very large quantities by the attackers, and (2) have a lower evaporation rate than other alarm pheromones, such as undecane and citronellal. Their active spaces thus reach a greater maximum radius, and they are more persistent. In a literal sense, the acetates operate as a form of "propaganda" directed at the victim colonies (Regnier and Wilson 1971). Propaganda would seem to be an effective alternative device to killing, which is the method resorted to by the highly specialized slave-making ants of the formicine genus *Polyergus.* The *Polyergus* workers have sickle-shaped mandibles that are superb combat weapons but useless in ordinary nest chores, and the slave-makers are completely dependent on their slaves for day-to-day existence. The workers of the *Formica sanguinea* group, in contrast, have "normal" mandibles and are able to assist in nest labor. As a result, they are far less dependent on slaves.

Finally, there is one additional ecological correlate which must be taken into account. All of the species known to be involved in the two major alterations of the alarm-defense system are also temporary social parasites. In temporary social parasitism, the queen establishes a colony by gaining entrance to a host colony of another species and usurping the position of the host mother queen. It is quite possible that the discharge of larger quantities of alarm-defense substances, some of them novel in structure, aids the queen in her penetration of hostile colonies. However, we doubt that this is the sole, or even the primary, function. In evidence is the fact that not all temporary parasites possess altered alarm-defense systems.

Parasitic members of the *F. microgyna* and *F. rufa* groups, for example, do not. More importantly, in the course of our studies we have been able to demonstrate important functions of the altered systems that are conducted by the worker caste. Such functions are quite separate from whatever role the secretions might play during colony founding.

SUMMARY

1. The higher formicine ants base their chemical alarm-defense systems primarily on a limited array of acyclic terpenes discharged from the mandibular glands, and alkanes and ketones discharged from Dufour's gland. All of these substances appear to be utilized in defense, and most, especially those at the lower end of the range of molecular weight (C_9—C_{13}), also function as alarm pheromones. The active space of the pheromones reaches over a distance of centimeters and is relatively short-lived. The alarm response of the various species can be classified roughly as either "panic" or "aggressive" in nature.

2. Two major adaptive alterations in the basic alarm-defense system have occurred within the higher Formicinae. In the genus *Acanthomyops,* the mandibular gland has been enlarged and made the site of storage of unusually large quantities of citronellal and two isomers of citral. We suggest that the changes are causally linked to the development of a strongly aggressive form of alarm communication. The response thresholds are also higher than in the one species (*Lasius alienus*) displaying a panic form of communication for which similar measurements have been taken. *Lasius* subgenus *Chthonolasius,* which is considered phylogenetically intermediate between *Acanthomyops* and the more primitive *Lasius* subgenus *Lasius,* also has an intermediate set of alarm-defense chemicals. *Lasius* subgenus *Dendrolasius* has evolved a system similar to that of *Acanthomyops,* except that cyclic terpenes (furans) are the substances produced in excess.

3. The second major adaptive change has occurred in certain species of the slave-making *Formica sanguinea* group. In these ants the Dufour's glands have been enlarged to become the site of large quantities of decyl, dodecyl, and tetradecyl acetates. The acetates are highly effective alarm substances, with extensive, long-lived active spaces. When discharged at target colonies during slave raids, they also alarm and help disperse the defending workers. Thus they serve both as a colonial signal and as an offensive form of "propaganda."

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